8200065

<u>aderenyaşıo zəhrakz deəhriyi şəhir</u>

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Fred K. Suzuki and Robert H. Dobberstein

Willievens, there has been presented to the

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED NOVEL VARIETY OF SEXUALLY REPRODUCED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY. AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO HE ENTITIED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

N()W, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT (S) AND APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF Eighteen YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT, OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IS A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCEPT OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IN THE PROPERTY OF THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT 142, AS AMENDED, 7 U.S.C. 2321 ET SEC.)

STEV1A

'P. J. Suzuki'

In Estimony Watercot, I have hereunto set my hand and caused the seal of the Plant Variety Protection Office to be affired at the City of Washington this 28th day of October in the year of our Lord one thousand nine hundred and sighty-two

hundred and eighty-two.

John Of 1

Sun Kenneth H.

Commissioner Plant Variety Grotection Office Gain Zivision

Agricultural Marketing Service

UNITED STATES DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE			FORM APPROVED OMB NO. 40-R3822			
APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE INSTRUCTIONS: See Reverse.			No certificate for plant variety protection may be issued unless a completed application form has been received (5 U.S.C. 553).			
Ia. TEMPORARY DESIGNATION OF VARIETY	Ib. VARIETY NAME		FOR OFFICIAL USE ONLY			
(PJS-1)	var. P. J. S	uzuki	8200065			
2. KIND NAME	3. GENUS AND SPE (CIES NAME	FILING DATE	TIME A.M.		
	 	7	2/2/82	2:00 P.M.		
Stevia	Stevia rebauc		FEE RECEIVED	DATE		
4. FAMILY NAME (BOTANICAL)	5. DATE OF DETI	ERMINATION	\$ 500.00	2/2/82		
Compositae	March 17, 1		\$ <u>250.00</u>	.8/16/82		
6. NAME OF APPLICANT(S)	7. ADDRESS (Street a	and No. or R.F.D. No.,	City, State, and ZIP	8, TELEPHONE AREA		
Fred K. Suzuki and Robert H. Dobberstein	746 West A	Algonquin Road Heights, Illinoi	is 60005	 (312) 593 - 0226		
9. IF THE NAMED APPLICANT IS NOT A P			D, GIVE STATE AND	11. DATE OF INCOR-		
organization: (Corporation, partnership	o, association, etc.)		PRPORATION	PORATION		
NA	NICANT DEPOSCENTAT	N A	CERVE IN THE ARRIVE	<u> </u>		
12. NAME AND MAILING ADDRESS OF APP ALL PAPERS:			SERVE IN THIS APPLI	ICATION AND RECEIVE		
Tom F. Prui tt,	_					
c/o Dr. Robert 746 West Algon 13. CHECK BOX BELOW FOR EACH ATTAC	auin_Road:_Arli	n <u>naton He</u> ight:	s <u>Illinois</u> 60	0005		
13A. Exhibit A, Origin and E	Breeding History of	the Variety (See Sect	tion 52 Of the Plant Va	riety Protection Act.)		
•r] 138. Exhibit B, Novelty Staten	ient.					
3C. Exhibit C, Objective Desc	cription of the Variet	y (Request form fron	n Plant Variety Protec	ction Office.)		
3D. Exhibit D, Additional Des	scription of the Varie	ty.				
	-	•				
148. DOES THE APPLICANT(S) SPECIFY THA SEED? (See Section 83(a). (If "Yes, " ansu			_	OT TO BE SOLD		
14b. DOES THE APPLICANT(S) SPECIFY THA LIMITED AS TO NUMBER OF GENERA		14c. IF "YES," TO 1	48, HOW MANY GENER BREEDER SEED?	RATIONS OF PRODUC-		
cl YES NO	1101137	FOUNDATION		CERTIFIED		
15a. DID THE APPLICANT(S) FILE FOR PROTECTION OF THIS VARIETY IN OTHER COUNTRIES? YES X NO (If "Yes," gir						
name of countries and dates,)			لسنا			
15b. HAVE RIGHTS BEEN GRANTED THIS V	ARIETY IN OTHER CO	OUNTRIES? YES	I if "Yes,"	'give name of countries		
and dates.)						
16. DOES THE APPLICANT(S) ACRES TO T	HE BURLLEATION OF	HICHER (THEIR) NA	ME(C) AND ADDRESS !	N THE OFFICIA L		
17. The applicant(s) declare(s) that a viable				application and will be		
replenished upon request in accordan	ce with such regulation	ons as may be applica	ble.			
The undersigned applicant(s) is (are)						
variety is distinct, uniform, and stable 42 of the Plant Variety Act.	as required in Sectio	n 41, and is entitled	to protection under the	e provisions of Section		
Applicant(s) is (are) informed that fa	lse represent :ation her	ein can jeopardize pr	otection and resulting	penalties.		
1/26/82		The	K /hu	and'		
(DATE)		<u> </u>	(SIGNATURE OF APP	LICANT)		
<i>V. A.</i> A. A.			SIGNATURE OF APPI Fred K. Suzukt			
136/82		Toler	FH. Nolla	erstens		
/ (DATE) FORM GR470 (1-78)		• 7	(SIGNATURE OF APPI ROBERT H. DOBB	LICANT) erstein a		

FORM GR470 (I-78)

1

ORIGIN AND BREEDING HISTORY OF Stevia rebaudiana Bert. var. P. J. Suzuki

The roots and seeds of *Stevia rebaudiana* Bert. used by applicants to develop the P. J. Suzuki strain were obtained (Morita Kagaku Kogyo Co., Ltd., Osaka, Japan) from plants growing in Hokkaido, Japan.

Root cuttings from three-year-old plants were shipped to the United States in January, 1974. The roots were planted in clay pots and stored in a cold-frame for one week, after which they were transferred to a green-house. Visible growth was apparent in February and growth was luxuriant by April. One hundred ten plants survived (ca.50%). In September 1974, root divisions were made to obtain additional plants with which to work. These root divisions yielded a total of approximately 485 plants. Seeds were collected from these plants in late September, but viability was poor (1-2%). After seed harvest, the plants were exposed to 14-hour day length by use of supplemental lighting with Gro-Light fluorescent bulbs. In October 1974, stem cuttings were made to yield an additional 1621 plants. These cuttings were made only to obtain additional plants for experimentation and were not part of the selection process.

Less than 2% of the seeds originally shipped from Hokkaido were viable.

A second lot of seeds from the same source were germinated in Jiffy peat

pots in February 1975, and grown in the greenhouse. Viability was still

poor (approximately 18.5%).

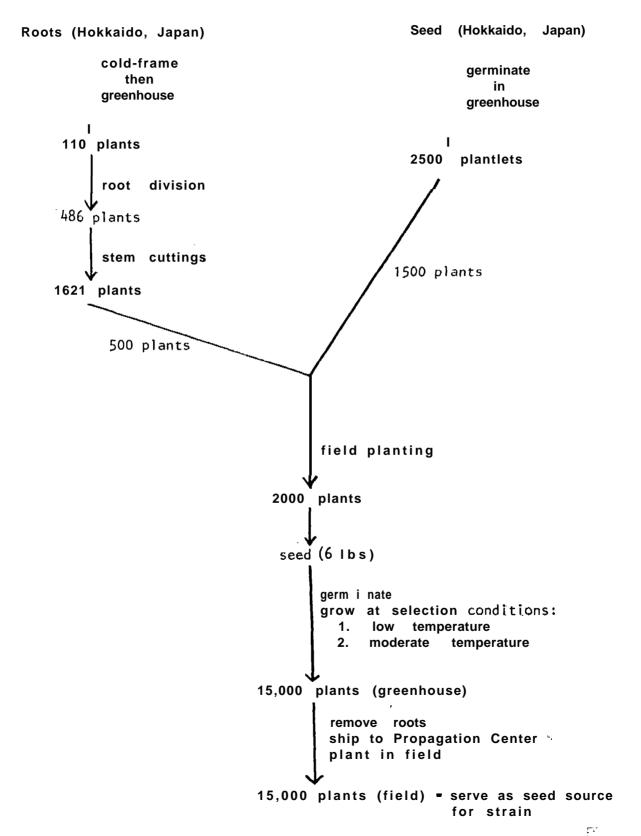
EXHIBIT A Page 2

In June 1975, 486 plants grown from the original root and stem cuttings were transplanted by hand into a field. At the same time 1500 plantlets from the seed germinated in February were transplanted by a mechanical transplanter into the field. Plants were irrigated as needed (I-4 times per month). Seeds (6.83 pounds) were collected from these field plants in September 1975.

All of this seed was germinated in Jiffy peat pots in an environmentally-controlled greenhouse at 24°C . Jiffy pots were placed in trays and incubated on electrically heated, thermostatically controlled, propagation mats—controlled at 33°C . Viability of this seed was 47-53%. When two true leaves had formed on most of the seedlings (approximately 2 weeks), the planting trays were removed and the greenhouse temperature was reduced to 21°C day temperature (14 hours) and 18°C night temperature (10 hours). Plant growth was poor at these low temperatures and only 42% of the plantlets survived. After 5 months the greenhouse temperature was raised to 24°C (day and night).

In September 1977, 15,000 roots of the plants (vide supra) were packed in moist sphagnum moss and shipped to the Company propagation site in California, where they were planted in the field. During the winters of 1977 to 1980, frost and flooding reduced the number of field plants by 25%. These plants were replaced by plants grown from stem cuttings of the field plants which survived. From 1979 to the present, the field plants served as a plant source for continued selection, as a continual source of seed of the described strain, and to obtain evidence of uniformity and stability. The variety has thus been proven to be stable and uniform.

SUMMARY OF ORIGIN AND HISTORY OF Stevia rebaudiana var P. J. Suzuki



INSTRUCTIONS

GENERAL: Send an original copy of the application and exhibits, at least 2,500 viable seeds, and \$500 fee (\$250 filing fee and \$250 examination fee) to U.S. Dept. of Agriculture, Agricultural Marketing Service, Livestock, Poultry, Grain and Seed Division, Plant Variety Protection Office, National Agricultural Library Building, Beltsville, Maryland 20705. (See section 180.175 of the Regulations and Rules of Practice.) Retain one copy for your files. All items on the face of the form are self-explanatory unless noted below.

ITEM

- Give the date the applicant determined that he had a new variety based on (1) the definition in section 41(a) of the Act and (2) the date a decision was made to increase the seed,
- Give: (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method; (2) the details of subsequent stages of selection and multiplication; (3) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified and (4) evidence of uniformity and stability.
- Give a summary statement of the variety's novelty. Clearly state how this novel variety may be distinguished from all other varieties in the same crop. If the new variety most closely resembles one or a group of related varieties:

 (1) identify these varieties and state all differences objectively; (2) attach statistical data for characters expressed numerically and demonstrate that these differences are significant; and (3) submit, if helpful, seed and plant specimens or photographs of seed and plant comparisons clearly indicating novelty.
- Fill in the Exhibit C, Objective Description form, for all characteristics for which you have adequate data.
- Describe any additional characteristics that are not described, or whose description cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the description of characteristics that are difficult to describe, such as, plant habit, plant color, disease resistance, etc.
- If "YES" is specified (seed of this variety be sold by variety name only as a class of certified seed) the applicant may NOT reverse his affirmative decision after the variety has either been sold and so labeled, his decision published, or the certificate has been issued. However, if the applicant specified "NO," he may change his choice. (See section 180.16 of the Regulations and Rules of Practice.)
- See section 42 of the Plant Variety Protection Act and section 180.7 of the Regulations and Rules of Practice.



Mr. Joseph J. Higgins April 7, 1982 Page 3

DITERPENE GLYCO	S. rebaudiana "Wild Type"	S. rebaudiana Var. P. J. Suzuki
Steviolbioside	0.04%	0.01-1%
Stevioside	2-10%	5-12%
Rebaudioside A	0.23-2.9%	3.7-6.9%
Rebaudioside B	0.04-0.07%	Less than 0.2%*
Rebaudioside C	0.4%	0.5-1.5%
Rebaudioside D	0.03%	Less than 0.2%"
Rebaudioside E	0.03%	Less than 0.2%*
Dulcoside A	0.19%	0.1-0.5%

The ordinate scale in Figure 2 is absorbance (optical density); the abscissa is time. Peak #3 is off-scale in Figure 2A but on scale in Figure 2B. The concentrations represented by the peaks are: Fig. 2A - peak 3 (steviosidej, 43.10 μg (8.62% of dry leaves weight); peak 5 (rebaudioside A), 11.26 μg (2.25% of dry leaves weight). Figure 2B - peak3, 30.15 μg (6.03% of dry leaves weight), peak 5, 24.14 μg (4.83% of dry leaves weight).

Table 2, "Exposure of Stevia rebaudiana var. P. J. Suzuki to adverse environmental conditions" was not a planned experiment, It represents field plants which were exposed to natural environmental changes. S. rebaudiana roots ("wild type") obtained from Hokkaido were propagated by root division and stem cuttings (Exhibit A, page 1). When plants from this source were grown in the field and exposed to similar natural environmental changes, their survival rate was 0%, compared to a survival rate of 85% for the P. J. Suzuki variety.

I have finally located references 21-47 as cited in our reference #19 and have enclosed them. I apologize for-their inadvertent omission in our original application.

We have completed the enclosed sunflower form to the best of our ability. However, some questions, such as "length of internode at harvest ripeness", and "number of leaves" are virtually impossible to answer since the plant is a perennial shrub which grows larger each year, and since the plant (and leaf) size is dependent on sunlight intensity and day length. In our experience, the morphilogical characteristics of the "wild-type" and the P. J. Suzuki variety are virtually indistinguishable. The only difference which we have observed is that the P. J. Suzuki variety plants grown in the same field show less plant to plant variation than do the wild type.

^{*}Concentrations were too low to accurately quantitate by the hplc methodology employed.

8200065

EXHIBIT B

NOVELTY -STATEMENT

Stevia rebaudiana Bert. var. P. J. Suzuki differs from other varieties of Stevia rebaudiana in:

- a. having a seed viability in excess of twenty-five percent (25%);
- b. having a rebaudioside A content in excess of three percent (3%) of dry leaf weight; and
- c. having a capability to survive after exposure to temperatures below $32^{\rm O}$ F for more than three (3) hours.

SUPPORTING INFORMATION

1. Historical Background

Stevia rebaudiana Bert is a wild shrub indigenous to Paraguay and possibly some contiguous areas in Brazil and Argentina (1). All though the plant was initially designated Eupatorium rebaudianium (2), it was later reclassified in the genus Stevia and renamed Stevia rebaudiana Bert. (3).

The sweet properties of this plant were first described scientifically in 1908 (4). The first sweet-tasting, pure, crystalline compound, stevioside, was isolated by Bride1 and Lavieille in 1931 (5-8). However, the complete chemical structure of stevioside was not elucidated until 1963 (9-16). Although early work suggested that stevioside was non-toxic (17), the plant has not been propagated on a large commercial scale for use as a sweetener because the seeds were largely sterile, such that reproduction by root cuttings was necessary (1, 10, 18, 19). This method of reproducing plants is both laborious and costly. In addition, the quantity of plants growing wild in Paraguay is not abundant (1). Although stevioside is being used as a sweetener in Japan today, its use is severely limited due to the paucity of high quality S. rebaudiana plant material available throughout the world.

To date, eight sweet diterpene glycosides have been isolated from.

S. rebaudiana. The names, chemical structures, and plant yields of these compounds are shown in Table 1.

TABLE 1. STEVIA REBAUDIANA BERT. (COMPOSITAE) DITERPENE GLYCOSIDES

8200065

COMPOUND	RELATIVE SWEETNESS	R ₁	R ₂	YIELD' (REFERENCE)
Steviol bioside	0.43	-Н	-H	0.04% (20)
Stevioside	1.00	-H	$-G^2^1G$	2-10% (1, 21)
Rebaudioside A	1.50	-G	$-G_3^2^1G$	0.23-2.9% (20-23) ²
Rebaudioside B	<1.50	-Н	¹ G - G ₃ ² - ¹ G	0.04-0.07% (20, 24)
Rebaudioside C (Dulcoside B)	0.10	-G	-G ² ¹ Rh	0.4% (25)
Rebaud ios ide D	1.0-1 .25	-g ² ¹ g	-G ₃ ¹ G	0.03% (25)
Rebaudioside E	1.00	-g ² ¹ g	-G ² ¹ G	0.03% (25)
Dulcoside A	0.25	- G	-G ² ¹ Rh	0.19% (26)

G = B-D-glucopyranosyl

 $Rh = \alpha - L - rhamnopyranosyl$

 $^{^{1}}$ Yield = percent of dry weight of leaves

This is the concentration of rebaudioside A in the original varieties of *S. rebaudiana*. The P. J. Suzuki: variety produces significantly larger amounts of rebaudioside A.

2. Di scuss ion of Novel ty

Stevia rebaudiana Bert. var. P. J. Suzuki is not anatomically distinct from the wild parent strain, although there is considerably less plant-to-plant variation in the appearance of the former strain.

The P. J. Suzuki strain is novel in three respects, seed viability, rebaudioside A content, and frost/flood resistance.

Seed Viability

As previously described, earlier strains of S. rebaudiana produced seeds which were largely sterile (1, 10, 18, 19). In our hands, seeds obtained from Paraguay consistently exhibited viability of only 1-2%. Initial seeds obtained from Hokkaido, Japan showed a viability of only 1.3%, although later samples exhibited increased viability, up to 18.5%. The P. J. Suzuki variety consistently produces seeds having a viability in excess of 25%. Seeds produced by the P. J. Suzuki variety can be up to 85-90% viable, with viability remaining approximately 30-60% after three years of storage. Seed viability of two seed samples from Hokkaido and the P. J. Suzuki variety are presented graphically in figure 1. The consistently high seed viability of the P. J. Suzuki variety makes it feasible for domestic cultivation as a source of sweet diterpene glycosides.

Rebaudioside A Content

Previous strains of S. rebaudiana produced stevioside as the major sweet diterpene glycoside (2-10% of dry leaf weight; references 1, 21) with rebaudiosi de A as a minor component (0.23-2.9% of dry leaf weight; references 20-23). When methanol extracts of different samples of the P. J. Suzuki variety, grown over a period of three years, were analyzed by high-performance liquid chromatography (27), they were consistently found to have a rebaudioside A content in excess of 3.0% of dry leaf weight. The P. J. Suzuki variety can have a rebaudioside A content of approximately 3.7 to 6.9% of dry leaf weight,

Figure 1. Germination Rate for Different Varieties of Stevia rebaudiana.

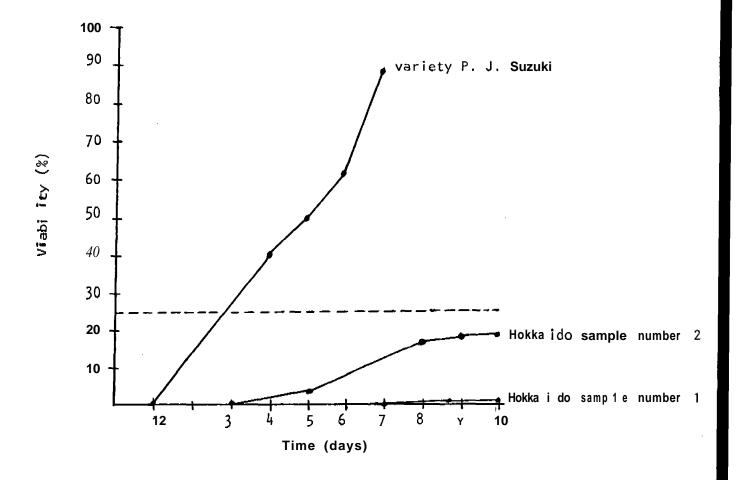


EXHIBIT B Page 6

and can also have a stevioside/rebaudioside A ratio of ca. 0.83-1.69. Thus, the rebaudioside A content of the P. J. Suzuki variety is ca. 27.6-2900% greater than that previously reported for S. rebaudiana. Since rebaudioside A is the sweetest and most pleasant tasting diterpene glycoside isolated from the plant, the advantage of the new variety is apparent. Typical high-performance liquid chromatograms of S. rebaudiana methanolic extracts from the Japanese and P. J. Suzuki varieties are shown in figure 2A and B, respectively, for comparison.

Frost/Flood Resistance

Wild strains of S. rebaudiana, e.g., plants grown from original root (Hokkaido) and stem cuttings, are very sensitive to frost and cannot survive more than short exposure (less than 2-3 hours) to temperatures below 32°F.

The P. J. Suzuki variety, however, shows marked resistance to freezing temperatures and flooding. It can survive after exposure to temperatures below 32° F. for more than 3 hours. Seven week-old P. J. Suzuki variety seed-1 ings, obtained by direct seeding in the field, were exposed to below freezing temperatures and flooding as shown in Table 2. These plants remained submerged under 4-5 inches of ice-water for one week. The survival rate for these seed-lings was approximately 85%, even though root development was poor due to plant immaturity. The data show that the P. J. Suzuki variety can survive three hours minimum daily exposure to low temperatures ranging from ca. 18° F to 31" F for at least seven to twelve days duration.

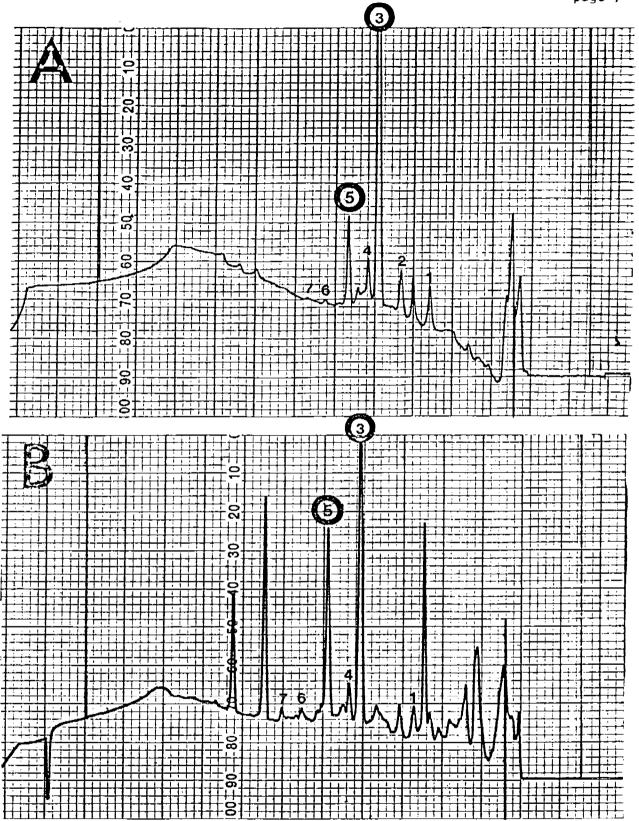


Figure 2 High-performance Liquid Chromatogram of Stevia rebaudiana Methanolic Extracts.

Operating conditions are a proprietary modification of those described in reference 27. A = Japanese plant variety; B = P. J. Suzuki variety. Peaks: 1 = steviolbioside; 2 = rebaudioside B; 3 = stevioside; 4 = rebaudioside C; 5 = rebaudioside A; 6 = rebaudioside E; 7 = rebaudioside D.

TABLE 2. EXPOSURE OF STEVIA REBAUDIANA VAR. P. J. SUZUKI TO ADVERSE ENVIRONMENTAL CONDITIONS

DATE	24-H0UI	RAINFALL (INCHES)	LOW TEMPERATURE (OF) *
January 2	28	0	31
February	2	0	29
February	6	0	27
February	8	1.20	35
February	9	1. 35	34
February	10	1.65	30
February	11	1. 32	26
February	12	2.06	22
February	13	1.00	19
February	14	1.15	20
February	15	0. 65	18
February	16	0.09	29
February	17	0	36

Low temperature which was maintained for at least three hours.

EXHIBIT C (Dahlia)

OBJECTIVE DESCRIPTION OF VARIETY

BANK 10 (DAME IA SPET) 10 14/29/82

		THE IDAI	-1 11-01-1-1 - (1-1) - (1-1)	,	
NAME OF APPL		II. Dahharatain	B.V.O.O.	FOR OFFICIAL USE ONLY	<u>, </u>
red K. Suzu	uki and Robert and No. of R.F.D. No., City, S	H. Dobberstein	PVPO	NUMBER 820006	5
	est Algonquin Road	stats, with zir code;	-VARIE	TY NAME OR TEMPORARY	
Arlingto		ois <i>60005</i>	DESIG Stevia	nation rebaudiana Bert.	var
Aimgu	on morgines, min	013 00000		J. Suzuki	vui.
	priate number that describes in first box (e.g. [8]9] or				
1. PLANT: (30	11.1		121/12		
		70-100 cm)	1 = COMPACT 2	= SPREADING	
2. MAIN STEM (At First Flower Cutting):	· · ·			
1 = STRA	IGHT 2 = FLEXUOUS/CR (DOKED			
Surface:	1= NOT GLAUCOUS 2=	GLAUCOUS 2	Surface: 1 = GLABR One to several	cous 2 = PILOSE heads per primary	branch,
0 5 NO. 01	head	BRXNEN (without disbudding		per head (heads ar	
			loosely panicu	late type of inflor	escence).
0 33 см. ве	TWEEN PRIMARY BRANCH	ES - variable depe	nding on light ex		C-5 1/26/82
2 0 MM. D	DIAMETER AT MID-POINT OF		GROUND - var i abl	C, 7 = 0 111111	rrangement
NA Flower to s	tem axis angle: 1 = < 30	2 = 30 0 - 60 0 3 =	60°- 90° of head	e due to type of a s in paniculate inf	lorescence
3. LEAF:					Er c
Type: 1	= SIMPLE 2 = PINNATE	3 = BIPINNATE	0.0		1/26/82
N A NO. OF	PINNAE PER LEAF		2410-FKS	1/26/82	
a 4 Shape:	1 = LANCEOLATE 2 = C	OVATE 3 = ELLIPTIC 4	= lanceolate-ob	1/26/82 long to elliptic-ob	lanceolate
a 5 Margin:	1 = ENTIRE 2 = DENTIC	ULATE 3 = DENTATE		<i>CHN, FES 16 62</i> entife at lower por serrate at upper po	
Texture:	1 ■ SOFT 2 = LEATHER	y 3 = chartaceous			16/82
Surface:	1 = NOT GLAUCOUS 2	= GLAUCOUS	2_{I} Surface: 1 = GLAB	ROUS 2 = PI LOSE ROUS / FKS / 2	6 182
4 Color:	1 = GREEN 2 E DARK (GREEN 3 = BRONZE (Pign	nented) 4 = OTHER (Sp	pecify) green to gray	<u>-green</u>
NA Rachis:	1 = NOT WINGED 2 = \	WINGED		POSITE QHD, FK	5 1/2//
NA Rachis:	1 = ROUND 2 = INVOL		EAF POSITION = OP	POSITE -	. 136/83
4. FLOWER:					
	PETIÖLE LENGTH BETWEEN	LAST LEAVES AND FLOWI	ER HEAD		
	1 = SINGLE	2 ■ ANEMONE	3 ■ COLLARETTE	4 = PEONY	
C 13 Type:	5 = FORMAL DECORATIVE	6 = DECORATIVE	7 = BALL	8 = POMPON	
	13 = salverform (p			r	م ابر
Size:	1 = TO 50 MM.	2 = 51 - 90 MM.	3 = 91 - 100 MM.	4 = 101 - 150 MM,	- A
	5 = 151 - 200 MMCACTU	16 = SONAFARGEIOTO MICVACTUS	11 = SEMICACTUS	12 = ORCHID	
4					41.
Ray Tips:	1 = POINTED 2 = NOTO	CHED 3 FIMBRIATE	4€ .,NO RAY	Soresan HO, AS	1/24/82

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
LIVESTOCK, POULTRY, GRAIN & SEED DIVISION
BELTSVILLE, MARYLAND 20706

(SUNFLOWER)

OBJECTIVE DESCRIP SMINELXIMERX X000FXX	TION OF VARIETY ANYXXIX Stevia rel	paudiana_
NAME OF APPLICANT(S) Fred K. Suzuki and Robert H. Dobberstein	variety name or P. J. Suzi	TEMPORARY DESIGNATION uki
ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code) 746 West Algonquin Road Arlington Heights, Illinois	DVOC NUMBER	oo 65
Place numbers in the boxes (e.g. [0] 8 19]) for the characters that best des	scribe typical $_{ m plants}$ of this variety. The symb	ool 🛦 indicates decimal.
COMPARISO	ON VARIETIEȘ	
NON-OIL: 1 = ARROWHEAD 2 = MINGREN 3 = SUND OIL: '4 = PEREDOVIK 5 = KRASNODARETS 6 = OTHE	I/ W	
1. CLASS:		
NA 1 = OIL TYPE 2 = NON-OIL TYPE (confectionery/		
2. MATURITY:SEE FOOTNOTE A	ŧ	
1 6 5 NO. OF DAYS TO HEAD FIRST VISIBLE (from emergence)	2 2 5 No. of days to harv	EST RIPENESS (from emergence)
N A DAYS EARLIER. THAN c I	N A DAYS EARLIER THAN	
HEADING SAME AS COMPARISON VARIETY	MATURITY SAME AS	COMPARISON VARIETY
N A DAYS LATER THAN'	N AAT AYS LATERTHAN	a
3. HEIGHT: SEE FOOTNOTE B		
7 5 cm TALL AT HARVEST RIPENESS	N A cm SHORTER THAN	c I
	SAME AS	COMPARISON C I VARIETY
	N A cm TALLER THAN	c 1
4. STEM:		
N A LENGTH OF INTERNODE AT HARVEST RIPENESS	N A AN UMBER OF LEAVES	
N A CM. SHORTER THAN	NAA EWER LEAVES THAN	
SAME AS a COMPARISON VARIETY	SAME AS	COMPARISON VARIETY
N A CM. LONGER THAN	N A MORE LEAVESTHAN	□)
BRANCHING:: 1 = NO BRANCHING 3 = TOP BRANCHING (with cents)	2 = BASAL'BRANCHI 4 = FULLY BRANCHE	NG, D (without central head)
COLOR OF GROWING POINT: 1 = GREEN 2 = YELL	LOW	″

ADDITIONAL DESCRIPTION OF THE VARIETY

From: Robinson, B. L. 1931. The Stevias of Paraguay. Con-t. Gray Herb. 90: 87.

"S. rebaudiana (Ber ton i) Hems 1. Suffruticose, erect, obscurely puberulent, 3-4.5 dm. high; leaves opposite, sessile, lance-oblong to spatulate-oblanceolate, obtuse, serrate above the middle, entire on the cunately narrowed base, of chartaceous or subcoriaceous texture, 3-nerved and conspicuously veiny, the cauline 3-5 cm. long, 7-15 mm. wide, often proliferous in axils; inflorescence becoming rather loosely paniculate, the heads (on pedicels often about as long as the involucre) appearing opposite the bracts in irregular sympodial cymes; corollas with pale purple throat and white limb; achenes nearly uniform, 15-17 aristate. -- Hemsl. in Hook. Ic. Pl. xxix. pl. 2816 (1906); Turrill, Kew Bull. 1918, p. 343-345 (1918). Eupatorium Rebaudianum Bertoni, Bol. Escuel. Agric. Asune. ii. 35 (1899).

Northern Paraguay: highlands of Amarobay and northward to the sources of the Rio Monday, Bertoni, Gosling (G r .).

This is the 'Sweet Herb of Paraguay.' Its leaves, even after drying, possess a singularly lasting sweet taste. The plant in consequence has been the subject of experimental cultures at Kew, at the Agricultural Station in Barbados, the Harvard Botanic Garden in Cuba, and doubtless at many other places. Although it is said to be quite free from noxious effects, no satisfactory methods have thus far been devised for its use as a sweetening agent in foods, preserves, etc. The plant, however, sti 11 remains a promising subject for further research and experimentation along these lines."

COMMENT BY APPLICANTS:

Stevia rebaudiana has plastic morphological characteristics. It has fibrous roots, usually white when young, becoming brown because of a cork layer.

The flower heads are homocarpic. The corolla is salverform (phlox-type) with five lobes. Figures 3 and 4 show line drawings and photographs of Stevia rebaudiana Bert, var. P. J. Suzuki.



April 7, 1982

Mr. Joseph J. Higgins
Examiner, Plant Variety
Protection Office
U.S. Department of Agriculture
Livestock, Poultry, Grain & Seed Division
National Agricultural Library Building
Bellsviile, Maryland 20705

RE: Stevia Application No. 8200065, "P. J. Suzuki"

Dear Mr. Higgins:

This is in response to the questions raised in your letter of February 23, 1982.

You are correct in assuming that the selections resulting in the P. J. Suzuki variety were largely based on survival at low temperatures. The increase in germination might be attributed to the crossing of plants from two different sources and/or to the low temperature selection process. The only plants retained were those which survived the low temperature treatment. These plants which survived the low temperature treatment exhibited all of the novel characteristics claimed for the P. J. Suzuki variety, i.e., these same plants produce seed with a higher germination rate, leaves with a higher rebaudioside A content, and show increased frost/flood resistance.

- As shown in Exhibit A, page 3, plants grown in the field were obtained from two sources:
 - 1. Plants produced by root division and stem cuttings from S.rebaudiana obtained from Hokkaido, Japan.
 - Plants grown from a second lot of seed of S. <u>rebaudiana</u> also obtained from Hokkaido, Japan and possessing a <u>viability</u> of approximately 18.5%.

Since plants from both sources were planted in the same field, some cross-pollination of the two plant sources undoubtedly occurred. All of the seed produced by the field plants was germinated (Exhibit A, page 2, paragraph 2). Only 47-53% of the seed was viable under the germination conditions used, which were selected to produce the highest rate of germination possible. When the resulting plantlets were approximately two-weeks-old, they were subjected to the selection conditions described in Exhibit A, page 2, paragraph 2.

Mr. Joseph J. Higgins April 7, 1982 Page 2

The surviving plants (42%) are-used as the continued seed source for the P. J. Suzuki variety. It was determined that the variety had been sexually reproduced with the recognized characteristics on March 17, 1980. A decision was made to increase the seed on this same date. The variety was named P. J. Suzuki on November 3, 1981. In the interim, the variety was designated as "PJS-1"

S. rebaudiana variety P. J. Suzuki has been examined through three successive sexual reproductive generations. No changes in morphilogical characteristics, seed viability, rebaudioside A content, frost/flood resistance, or other measurable characteristics were observed. Thus the variety has been shown to be stable.

Our use of the term "other varieties" of <u>S. rebaudiana</u> in Exhibit B was perhaps misleading. To the best of our knowledge, no discrete strains or varieties of <u>S. rebaudiana</u> have been previously identified. We used the term "other varieties" to indicate <u>S. rebaudiana</u> plants which have previously been described in the literature, which are presumably wild types.

Novel characteristics of the P. J. Suzuki variety are as follows:

CHARACTERISTIC	<u>S. rebaudiana</u> Wild Type	S. <u>rebaudiana</u> Var. P. J. Suzuki
Seed viability	1-2%	85~90%
Rebaudioside A content (% dry weight of leaves)	0.23-2.9%	3.7-6.9%
Frost resistance (Survival temperature/ times)	Less than 2-3 hours. at 32°F or below for 1 day.	More than 2-3 hours. at 18-31°F for at least 7-12 days.

We consider the normal germination rate for P. J. Suzuki variety seeds to be approximately 87%. When the "wild type" seed is germinated under identical conditions the viability rate is approximately 1.5%. The data shown in Figure 1 for Hokkaido sample number 1 is representative of the wild type as described in the literature. Although Hokkaido sample number 2 does not represent a named variety, the germination rate for this seed (approximately 18.5%) is significantly higher than that previously reported in the literature,

Previous "strains" of S. rebaudiana, i.e., plants previously reported in the literature, produce $0.\overline{23-2.9}$ rebaudioside A, whereas P. J. Suzuki variety produces $3.7 \sim 6.9$ % rebaudioside A. This represents a difference of 27.6% (3.7-2.9) χ 100) to 2.900% (3.900% (3.900% x 100) and is surely significant. Reference 27.2% was cited to illustrate the methodology used for high-performance liquid chromatographic analyses. The results of the analyses described in this reference were for "wild type" plants, not the P. J. Suzuki variety. For comparative purposes, the diterpene glycoside content of S. rebaudiana previously reported in the literature ("wild type") and P. J. Suzuki are as follows:

FORM GR-470-24 (Rever	logo	0,000 - 00
4. FLOWER (Continued		
N A Ray Color: s	e Ic tfrom colors below. Consider only the predominant colors on the pall inflorescences in which the reverse of the involute rays dominate.	face of the rays except for those pompon or (See References below.)
N A MONOCOLO	R Select from colors below.	
Pattern:		
GROUND CO	DLOR DOT FLECK	SPLASH
NARROW LI	NE PICOTEE NAA	Specify) NOT APPLICABLE
Disc Flower:		
2 1 = ABSENT	2 = PRESENT MONOC	OLOR
0 1 PRIMARY C	OLOR 0 9 SECONI	DARY COLOR - COTOLIA throat usually portion
O1 = WHITE	02 = YELLOW	bitan
06 = RED		PURPLE 10 BRONZE
11 = FLAME 12		THER (Specify)
5. DISEASE RESISTANC	CE (0 = Not Tested; 1 = Susceptible; 2 = Resistant)	
C 0 MOSAIC	C RINGSPOT OTHER (Sp.	ecify)
6. INSECT RESISTANCE	(0 = Not Tested; 1 ≈ Susceptible: 2 = Resistant)	
1 APHID PE	S 1/36/83 THRIP O BORER	O OTHER (Specify)
7. INDICATE A VANTA	THE THAT MOST CLOSELY RESEMBLES THAT SUBMITTED:	841 PXS 1/26/82
CHARACTER	SPECIE XXXXXX QHO, AS CHARACTER	SPECIE XXXXXXX
	tevia ammotropha Robinson Flower size	UNKNOWN
	tevia amambayensis Robinson Flower shape	
Leaf shape S_{0}	tevia ammotropha Flower color	Stevia entreriensis
	REFERENCES	

RAY COLOR: Described in the 1971 Classification of Dahlias compiled and published by the Joint Classification Committee sponsored jointly by the American Dahlia Society, Inc. and the Central States Dahlia Society, Inc.

American Dahlia Society. Current Series. <u>Classification of Dahlias.</u>

Barnes, A.T., 1966. The Dahlia Grower's Treasury. Collingridge, London.

Cook, W. H., 1953. Judging Dahlias in Shows. Amer. Dahlia Society

U.S.D.A. Home and Garden Bul. 131, 1970. Growing Dahlias.

COMMENTS:



AMS, LPG&S DIV. PVP0

EXHIBIT C (SUNFLOWER)

U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE LIVESTOCK, POULTRY, GRAIN & SEED DIVISION BELTSVILLE, MARYLAND 20705

OBJECTIVE DESCRIPTION OF VARIETY

SMNELXIVEEX XIVEX NA	NXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
NAME OF APPLICANT(S) Fred K. Suzuki and Robert H. Dobberstein	variety name or temporary designation P. J. Suzuki
ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code)	FOR OFFICIAL USE ONLY
746 West Algonquin Road Arlington Heights, Illinois	60005 82 000 6 5
Place numbers in the boxes (e.g. [0] 8 [9]) for the characters that best desc	cribe typical plants of this variety. The symbol 🛕 indicates decimal.
COMPARISO	N VARIETIES
NON-OIL: 1 = ARROWHEAD 2 # MINGREN 3 = SUNDAI OIL: 4 = PEREDOVIK 5 = KRASNODARETS 6 = OTHER	IV A
. CLASS:	
NA 1 = OIL TYPE 2 = NON-OIL TYPE (confectionery)	
MATURITY:SEE FOOTNOTE A I 6 5 ND. OF DAYS TO HEAD FIRST VISIBLE (from emergence)	2 2 5 NO. OF DAYS TO HARVEST RIPENESS (from emergence)
N AAD A Y S EARLIER THAN C I	N A DAYS EARLIER THAN c
HEADING SAME AS C I COMPARISON WAR I ETY	MATURITY SAME AS COMPARISON VARIETY
N A ADAYS LATER THAN	N AADAYS LATERTHAN a
HEIGHT: SEE FOOTNOTE B	
7 5 cm TALL AT HARVEST RIPENESS	N A cm SHORTER THAN
	SAME AS COMPARISON VARIETY
	N A cm TALLER THAN
4. STEM:	
LENGTH OF INTERNODE AT HARVEST RIPENESS	N AA NUMBEROF LEAVES
N A CM. SHORTER THAN	N A FEWER LEAVES THAN
SAME AS COMPARISON a VARIETY	SAME AS COMPARISON VARIETY
N A CM, LONGER THAN C I	N A MORE LEAVESTHAN C I
BRANCHING: 1 = NO BRANCHING 3 = TOP BRANCHING (with central)	2 = BASAL BRANCHING 4 = FULLY BRANCHED (without central head)
COLOR OF GROWING POINT: 1 = GREEN 2 = YELLO	ow wc

5. LEAVES (Mi	determ at flowering):
5 7	CM. BLADE LENGTH SEE FOOTNOTE A
N A	CM. SHORTER THAN
	LENGTH SAME AS COMPARISON WIDTH SAME AS COMPARISON VARIETY
N A	CM. LONGER THAN
_ <u>1</u> v	WIDTH: LENGTH RATIO: 1 = NARROWER THAN LONG 2 = EQUAL 3 = WIDER THAN LONG
2	LEAF SHAPE: 1 = CORDATE 2 = OTHER = lanceolate-oblong to elliptic-oblanceolate
c ² 1	LEAF APEX: 1 = ACUMINATE 2 = OTHER Obtuse
	LEAF BASE: 1 = AURICULATE 2 = TRUNCATE 3 = cuneately narrowed entire at lower
4	LEAF MARGIN: 1 = ENTIRE 2 = FINELY CRENATE 3 = COARSELY CRENATE 4 = OTHER portion, serrate at upper portion
cl 1	DEPTH OF MARGIN INDENTATIONS: 1 = SHALLOW 2 = INTERMEDIATE 3 = DEEP
EI 2	ATTITUDE: 1 = ERECT 2 = ASCENDING 3 = HORIZONTAL 4 = DESCENDING
ci 1	SURFACE: 1 = SMOOTH 2 = CRINKLED (ridged) 3 = OTHER
5	COLOR: 1 = LIGHT GREEN 2 = GREEN 3 = DARK GREEN 4 = BROWN 5 = green to gray-green
3	MARGIN COLOR: 1 = GREEN 2 = YELLOW 3 = green to gray-green
6. HEAD AT	FLOWERING:
1	RAY FLOWERS: 1 = ABSENT 2 = PRESENT
NA	RAY FLOWER COLOR: 1 = YELLOW 2 = SULFUR YELLOW 3 = ORANGE YELLOW 4 = OTHER
c^4	DHSK FLOWER COLOR: 1 = YELLOW 2 = RED 3 = PURPLE 4 = white, usually with purplish corolla throa
c l	ANTHOCYANIN IN STIGMAS: 1 = ABSENT 2 = PRESENT
E 1	POLLEN COLOR: 1 = WHITE (colorless) 2 = YELLOW
3	PAPPI: 1= GREEN 2 = RUST (red) 3 = white, becoming straw-colored as achene matures
N A	MM, RAY LENGTH N A MM. RAY WIDTH
N A	MM. SHORTER THAN NARROWERTHAN
	SAME AS L - I COMPARISON VARIETY SAME AS COMPARISON VARIETY
N A	MM. LONGERTHAN MM. WIDER THAN
·	

'HEAD AT SEED MATURITY:
CM. DIAMETER
NA CM. NARROWER THAN
SAME AS COMPARISON VARIETY a
N A CM. WIDER THAN
1 RECEPTACLE SHAPE: 1 = FLAT 2 = CONVEX 3 = CONCAVE
a] HEAD ATTITUDE: 1 = VERTICAL (erect) 2 = ASCENDING 3 HORIZONTAL 4 = DESCENDING
NO. OF SEEDS PER HEAD
N A SEEDS / HEAD LESS THAN
SEEDS / HEAD SAME AS COMPARISON VARIETY
N A SEEDS / HEAD MORE THAN
. SEEDS:
OUTER PERICARP: 1 = CLEAR 2 = STRIPED BLACK 3 = NEARLY SOLID BLACK 4 = light brown longitudinerves
a 3 MIDDLE PERICARP: 1 = WHITE 2 = SOLID PURPLE 3 = dark brown
c 31 INNER PERICARP (seed coat): 1 = NO COLOR 2 = BROWNISH BLACK 3 = white
5 STRIPES: 1 = ABSENT 2 = EVEN BLACK & WHITE STRIPES 3 = BROAD BLACK & NARROW WHITE
4 = BLACK WITH NARROW DARK-GREY STRIPING 5 = OTHER lighter brown longitudinal_stripes or nerves
C 1 MOTTLING: 1 = ABSENT 2 = PRESENT
6 = oblong-acicular 6 = oblong-acicular Shape: 1 = Ovate 2 = Obovate (shield) 3 = NARROWLY OBOVATE 4 = Oblong 5 = ELLIPTIC with somewhat narrowed base
2 SHAPE (cross-section): 1 = NOT CURVED 2 = CURVED
3 10 MM, LENGTH $3 10$ GM. / 100 SEED 0.04 gm/ 100 seeds
N A MM. SHORTERTHAN a N A GM. LIGHTER THAN
SAME AS COMPARISON SAME AS COMPARISON VARIETY COMPARISON
N A MM, LONGER THAN C I N A GM, HEAVIER THAN a
N A % HELD ON 7.9 MM. (20/64) ROUND-HOLE SCREEN
NA A % LESS THAN
SAME AS COMPARISON VARIETY
N A % MORE THAN

9. DISEASE AND INSECT	S (0 = Not tested, 1	■ Susceptible, 2 = Re	esistant):							
 -	(Puccinia helian thi)				0 SCLER	OTI NI A	WILT (Scie l	rotinia :	sclerotioru	ım)
	RACES:	rticillium dahliae)		_	c / LEAF	MOTTLE	IV. <i>albo-ati</i>	rum)		
<pre>0 DOWNY MILDEW (Plasmorpara halstedii) a</pre>				C / GRAY-N	IOLD BI	LIGHT, BUD	ROT	(Botrytis	cinerea)	
0 WHITE	BLISTER RUST (A	l bug o tragopogi)			0 CHARCO	DAL ROT	, STEM RO	T (Mac	rophomin	a phaseolina
ت ا	M RAPE (Orobanche /E RACES:	cannis)		_	بنا		OTH, N. AI electellum)	MERICAI	N HEAD	мотн
c / EUROP	PEAN SUNFLOWER I	мотн <i>(H. nebullela)</i>			OTHER	(specify)				
c / OTH E	R (specify)			_	OTHER	(specify))			
	esemblance by placing	MOST CLOSELY R g in the <i>column</i> marked ferior than comparison	J, D.R., one	THE SUBMI of the folloge 2 = Same as	wing numbers:		e following o			CABLE
CHARACTER	V.	ARIETY	D.R.	СНА	RACTER		VARIET	Υ		D.R.
Frost resistance				Leaf attitue	de					2
Lodging resistance				Heed attitu	de					-2
Neck or stem strength				Ray flower	color					
Branching type			2	Seed shape	1					2
Petiole length				Seed color						
Leaf shape			2	Seed stripi	ng pattern					2
Leaf color (green)				Seed yield						
11. GIVE THE FOLLOW	VING DATA FOR SU	IBMITTED AND A SI	MILARVA	RIETY*.	NOT APPLI	CABLE				
				(5/)			F	ATTY	ACIDS	
VARIETY	HULL (%)	PROTEIN (%)	OIL	(%) IODINE NO.						EIC (%)
Submitted										****
Similar										
Name of similar variety										

• Hull, protein and oil percentages expressed for whole undecorticeted seed; acids expressed es percentages of oil

1 2 . COMMENTS:

Stevia rebaudiana vax. P. J. Suzuki, like the S. rebaudiana "wild type", is a perennial in mild climates. It is a short day plant with somewhat plastic morphological characteristics highly dependent on plant age, day length, and light intensity, as is the "wild type".

